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Comparison of negative inotropic potency, reversibility, and effects on calcium influx of six calcium channel antagonists in cultured myocardial cells

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1 The negative inotropic effects of calcium channel antagonists on the myocardium were used as a standard for the definition and determination of potency of this group of drugs.

2 The effects of six calcium channel antagonists (verapamil, methoxyverapamil (D600), nifedipine, lidoflazine, perhexiline and diltiazem) were compared on cultured chick embryo ventricular cells.

3 Drug concentrations producing 50% inhibition of contractile amplitude, derived from linearized concentration-response curves, varied from 2.8×10^{-8}M for nifedipine to 8.3×10^{-7}M for perhexiline. Equipotent negative inotropic concentrations of verapamil, D600, perhexiline, diltiazem and lidoflazine produced a similar inhibitory effect on ^{45}Ca uptake into cultured cells. Nifedipine produced no significant inhibition of ^{45}Ca uptake.

4 The time required for recovery of contractility after cessation of drug superfusion varied in the order lidoflazine > perhexiline > D600 > verapamil > nifedipine > diltiazem. This relative order accords closely with the reported *in vivo* half-lives of these drugs.

5 It is concluded that while some inhibition of $^{45}Ca^{2+}$ uptake into cardiac cells can be demonstrated with five of the six calcium channel blockers studied, the relationship between the degree of inhibition of calcium influx and negative inotropic effects may not be uniform for all calcium channel antagonists.

Introduction

A number of drugs developed in recent years for the management of myocardial ischaemia, cardiac arrhythmias and hypertension have been shown to inhibit the influx of calcium into vascular smooth muscle and myocardium. These agents have therefore been designated calcium channel antagonists. However, among the large number of drugs that may inhibit calcium uptake into certain tissues under specific circumstances, considerable heterogeneity of *in vivo* effects has been observed, due in large part to variations in the relative sensitivity of myocardium and vascular smooth muscle to their effects.

In view of the complexity of effects of these agents on vascular smooth muscle, they have generally been characterized on the basis of inhibitory effects on calcium influx via the voltage sensitive 'slow channel' into myocardial cells (Fleckenstein, 1971; 1977; Nayler & Poole-Wilson, 1981; Zelis & Flaim, 1981). However, even within isolated myocardial preparations, difficulties are frequently encountered in comparing the relative potencies of the various calcium channel antagonists. While for many of these drugs it might be expected that there would be a relatively close correlation between their negative inotropic effects and inhibition of calcium influx via the slow channel, such a correlation has hitherto proved difficult to demonstrate (Nayler & Poole-Wilson, 1981). There are several reasons for this difficulty. First, slow channel activity may account for only a small proportion of total calcium influx (Barry & Smith, 1982). Second, many of the calcium channel antagonists exert

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additional pharmacological actions that may modify their inotropic effects in isolated cardiac preparations (Galper & Catterall, 1979; Vaughan Williams, 1980; Fleckenstein & Fleckenstein-Grün, 1980; Jim *et al.*, 1981). Indeed, Williams & Jones (1983) have reported specific high-affinity binding of the dihydropyridine calcium channel antagonist, nitrendipine, to a myocardial cell fraction enriched in sarcoplasmic reticulum, while binding did not correlate with sarcolemmal markers, suggesting that effects of this class of calcium channel antagonists may be exerted at a site or sites other than the sarcolemmal slow calcium channel. Finally, it has proved difficult to demonstrate, let alone quantify, inhibition of calcium uptake into myocardium by calcium channel antagonists (Nayler & Poole-Wilson, 1981), except by inference using observations of slow inward current and action potential plateau characteristics (Kass & Tsien, 1975; Kohlhardt & Fleckenstein, 1977).

We have used a cultured myocardial cell preparation to compare the negative inotropic effects of six commonly used calcium channel antagonists and to assess the rate and extent of reversibility of the effects of the various agents examined. We also sought to determine if equipotent negative inotropic concentrations of these drugs produce a comparable degree of inhibition of rapid unidirectional influx of ^{45}Ca (Barry & Smith, 1982).

Methods

Tissue cultures

Monolayer cultures of contracting chick embryo ventricular cells were prepared as previously described (Barry *et al.*, 1981). Briefly, fragments of ventricles from 10-day-old chick embryos were removed aseptically and tissue fragments placed in Ca and Mg-free Hanks' solution. The cells were isolated by 4 cycles of trypsinization with 0.025% w/v trypsin at 37°C. Cell suspensions from each dissociation cycle were placed in 20 ml of cold trypsin inhibitor solution (50% heat-inactivated foetal calf serum and 50% Ca and Mg-free Hanks' solution). The resultant cell suspension was centrifuged at 1000 r.p.m. for 10 min and the supernatant discarded. The pellet was resuspended in culture medium consisting of 6% heat-inactivated foetal calf serum, 40% medium 199 with Hanks' salts, 0.1% penicillin-streptomycin solution, and 54% balanced salt solution containing, in mM concentrations: NaCl 116, NaH_2PO_4 1.0, $MgSO_4$ 0.8, KCl 1.18, $NaHCO_3$ 26.2, $CaCl_2$ 0.87 and glucose 5.5. Final concentrations (in mM) in the culture medium were Na^+ 144, K^+ 4.0, Ca^{2+} 0.97, Mg^{2+} 0.8, Cl^- 131 and HCO_3^- 18.

The cell suspension was diluted to 5×10^5 cells ml^{-1} and placed in plastic Petri dishes containing 25 mm circular glass coverslips. Cultures were incubated in a humidified 5% CO_2, 95% air atmosphere at 37°C. Confluent monolayers in which at least 80% of cells exhibited spontaneous synchronous contractions developed by 3 days, at which time the experiments were performed.

Measurement of contractility

Changes in the contractile state of individual cells in the monolayers were quantified by the use of an optical-video system as previously described (Barry *et al.*, 1981). Briefly, a glass coverslip with attached ventricular cell monolayer was continuously superfused in a chamber provided with inlet and exit ports. The chamber was positioned on the stage of an inverted phase microscope. The apparatus was enclosed in a heated Perspex (Lucite) box and maintained at a temperature of 37°C. The cells were magnified with a 40 × objective, and the image monitored by a low light level TV camera connected to a video motion detector that monitored a selected raster line segment and provided position data every 16 ms for an image border moving along that raster line. The analogue voltage output from the motion detector was calibrated to indicate actual μm of cell motion. The first derivative was obtained electronically and recorded as velocity of motion in $\mu m\,s^{-1}$.

Media bathing the cells were infused by Harvard syringe pumps at a flow rate of 0.95 ml min^{-1}. Cells were perfused with culture medium containing 0.97 mM Ca^{2+}. All drugs used were diluted at least 100 fold from stock solution in culture medium containing 0.97 mM Ca^{2+}.

Inotropic effects were quantified by measuring the amplitude of contraction of the observed cells. Concentration-effect curves for the negative inotropic effects of the various calcium channel antagonists examined were obtained by superfusing various concentrations of the drugs for periods of time sufficient to reach steady-state negative inotropic effects, before increasing the concentration of the superfused drug. In the cases of verapamil, nifedipine, diltiazem and methoxyverapamil (D600), steady-state drug effects were reached within 5 min of the start of superfusion of any given drug concentration; for lidoflazine and perhexiline 7 min periods were required.

Studies of the extent and time course of reversibility of the negative inotropic effects were performed after initial determination of concentration-effect curves for each drug examined. Myocardial cells were superfused with the minimal concentration of each drug sufficient to abolish spontaneous contractions. Immediately following cessation of contraction, superfusion of drug was stopped, drug-free culture medium was superfused, and amplitude of cellular contraction

was recorded for up to 20 min during this washout phase, or until the amplitude of cellular contraction returned to 90% of its control value.

Calcium fluxes

Determination of drug effects on Ca uptake by cultured ventricular cells was made by means of previously described techniques (Barry & Smith, 1982). Approximately 140 glass coverslips with attached monolayers were prepared from each culture. The cells were exposed to [^{3}H]-leucine (0.2 μCi ml^{-1}, [leucine] = 28 mg l^{-1}) for 24 h before their use in uptake experiments. [^{3}H]-leucine was incorporated into cell protein, and subsequent determination of ^{3}H counts in relation to protein measurements by the methods of Lowry *et al.* (1951) permitted normalization of ^{45}Ca content relative to mg of cell protein for each coverslip.

For measurement of the ^{45}Ca uptake rate, coverslips were loaded in groups of 8–10 onto small Perspex (Lucite) baskets. After equilibration for 30 min in tissue culture medium at 37°C in a 5% CO_2 atmosphere, cells were preincubated in the desired concentration of calcium channel antagonists for 5 min (for verapamil, D600, nifedipine and diltiazem) or 7 min (for perhexiline and lidoflazine). The baskets were then transferred to culture medium containing ^{45}Ca (5 μCi ml^{-1}, total [Ca] 0.6 mM) as well as the previous concentration of calcium channel blocking drug, in a 5% CO_2 atmosphere at 37°C. After the desired uptake period, ranging from zero to 100 s, coverslips were removed from the uptake medium and washed for 16 s in ice cold balanced salt solution.

After washing, the monolayer was scraped off the coverslip and the cells were dissolved in 1% SDS–0.01 M sodium borate. Dissolved cells were resuspended in liquid scintillation fluid, and simultaneous counting of ^{45}Ca and ^{3}H counts was performed using a Packard liquid scintillation spectrometer. Uptake curves were calculated using n of ≥ 8 for each time point, and the data fitted to the equation $[Ca] = R/K\,(1\text{-}e^{-Kt})$ where R is the rate of uptake, K is the efflux rate constant, and t is time in min (Barry & Smith, 1982).

In order to compare the effects on ^{45}Ca influx of concentrations of calcium channel antagonists that exerted equivalent negative inotropic effects, drug concentrations associated with 90% contractility inhibition were derived from linearized dose-response curves (see below) and were used in these experiments. In all cases, because the control magnitude of Ca uptake varied somewhat from culture to culture, and because only 3 to 4 conditions could be studied per culture, the inhibitory effects of other calcium channel blocking drugs on ^{45}Ca influx were compared with those of verapamil, which was used in all experiments and which produced a consistent and statistically significant 16 to 24% decrease in rapid Ca uptake (Barry & Smith, 1982).

Statistical methods

Effects of drugs on mean amplitude of cell motion were expressed as percentage reductions (relative to initial amplitude). For each point in the concentration-effect curve, a minimum of 7 cells on different coverslips from at least two separate cultures was examined. Concentrations producing 50% (EC_{50}) and 90% (EC_{90}) reduction in amplitude of cell motion were determined after linearization of the concentration-effect curves by the logit transformation method (Armitage, 1971). Drug concentrations corresponding to EC_{90} values were used in ^{45}Ca uptake experiments. Mean times for recovery of spontaneous contractility after drug washout were determined from linear regression plots of the central segment of recovery of the contractility-time curve for these drugs.

Analysis of the rapid phase of ^{45}Ca uptake was accomplished by a modification of the method of Feldman (1977) as described by Barry & Smith (1982). The rates of ^{45}Ca uptake, R, in the absence and presence of drugs were determined. Inhibitory effects of other calcium channel blocking drugs on rates of ^{45}Ca uptake were compared with the effects of the EC_{90} concentration of verapamil (3.0×10^{-7} M) in each experiment.

Drugs

The following drugs were used: verapamil hydrochloride (Knoll), D600 hydrochloride (Knoll), dil-

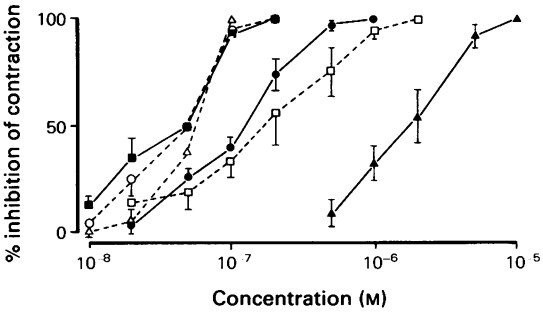

Figure 1 Concentration-effect curves for the six calcium channel blocking drugs examined. Each point represents the mean of at least six observations. To preserve clarity, some of the standard error bars have been omitted. Symbols used for each drug are as follows: (●) verapamil; (■) nifedipine; (▲) perhexiline; (○) D600; (□) diltiazem; (△) lidoflazine.

tiazem hydrochloride (Marion), lidoflazine (Janssen), nifedipine (Pfizer), and perhexiline maleate (Merrell-Dow). Stock solutions of all drugs were made up at concentrations of 10^{-3}M in 100% ethanol; subsequent dilutions (of at least 100 fold) were made in culture medium. Preliminary experiments showed that concentrations of ethanol of up to 1% had no effect on the contractility of the preparation. Nifedipine was protected from exposure to light during weighing, and syringes and perfusion tubing used in contractility experiments were wrapped in foil. During ^{45}Ca uptake studies, uptake chambers were also shielded from light. Nifedipine and diltiazem solutions were prepared from crystalline material for each experiment; other drugs were stable on storeage in 100% ethanol at −30°C for up to 4 weeks. ^{45}Ca and [^{3}H]-leucine were obtained from New England Nuclear, Boston, Mass.

Results

Effects on contractility

Amplitude of contraction of cells was stable under control conditions for at least 60 min. For studies of the effects of calcium channel blocking drugs on amplitude of contraction, control amplitude of cell motion was recorded over a 10 min period. Cells were then superfused with graded concentrations of calcium channel antagonists, and measurements repeated when steady-state effects were attained. All agents examined produced progressive reductions in amplitude of cell motion, without significant changes in beating rate until a greater than 90% reduction in amplitude of contraction had occurred.

Table 1 Characteristics of logit transformed concentration-effect curves for negative inotropic effects of calcium channel blocking drugs

Drug	EC_{50}(M × 10^{-8})	EC_{90}(M × 10^{-8})
Nifedipine	2.8 (0.8–7.8)	7.1 (3.5–14.4)
D600	3.8 (3.0–4.8)	8.4 (6.6–10.7)
Lidoflazine	4.3 (1.4–12.9)	7.6 (3.7–15.6)
Verapamil	10.0 (8.2–12.0)	30 (24–37)
Diltiazem	13.5 (8.5–20.4)	52 (31–87)
Perhexiline	83 (63–109)	480 (250–920)

Data were derived from the concentration-effect curves shown in Figure 1. Minimum correlation coefficient for linearity of the transformed data was 0.94.
95% confidence limits are given in parentheses.

The potency of the six calcium channel antagonists in reducing contractility varied widely, as can be seen from their concentration-effect curves (Figure 1). In each case, the transition from threshold of detectable drug effect to complete inhibition of spontaneous contraction occurred within approximately a 10 fold range of drug concentration. Logit transformation of these concentration-effect curves revealed no significant differences in the slope of the linearized responses. However, EC_{50} values ranged from 2.8×10^{-8}M for nifedipine to 8.3×10^{-7}M for perhexiline; all of the other drugs examined were significantly more potent than perhexiline on a molar basis (Table 1).

In view of the instability of nifedipine on exposure to light, this drug was protected in all experiments by covering syringes and tubing with aluminium foil.

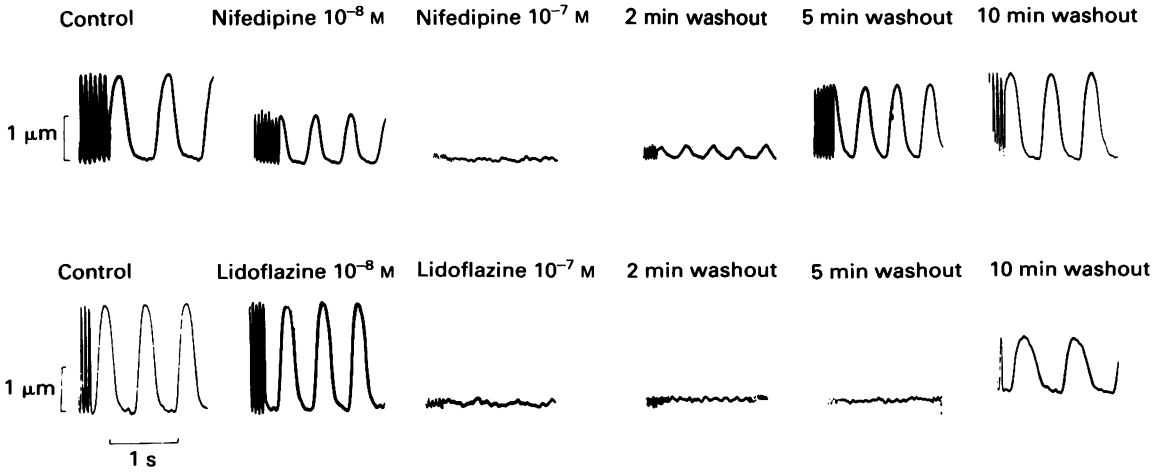

Figure 2 Recordings showing the difference in time course of recovery following washout of nifedipine 10^{-7}M and lidoflazine 10^{-7}M.

Table 2 Time course of reversibility of negative inotropic effects of calcium channel blocking drugs, compared with estimated plasma half-lives in man from the literature

Drug	*Concentration superfused (M)*	R_{50} (min)	*Plasma* $t_{1/2}$ (h)	*Reference*
Diltiazem	10^{-6}	3.0 (2.3–3.7)	4.1[a]	Zelis & Kinney (1982)
Nifedipine	10^{-7}	3.2 (2.7–3.7)	2.9	Rämsch & Sommer (1977)
Verapamil	5×10^{-7}	4.5 (3.8–5.2)	7.5[a]	Freedman *et al*, (1981)
D600	10^{-7}	7.4 (6.9–7.9)	NA	
Perhexiline	10^{-5}	16.3 (15.2–17.4)	11.2[9]	Horowitz *et al*, (1981)
Lidoflazine	10^{-7}	16.8 (12.4–21)	21	Heykants *et al*, (1981)

R_{50} is the time for return to 50% of control amplitude of contraction (mean and 95% confidence limits are given).
NA = no available information.
[a] Evidence for saturable metabolism

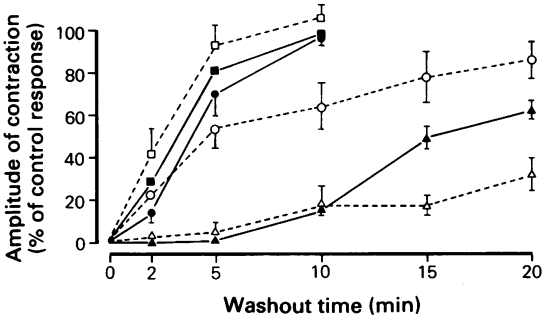

Figure 3 Time course of recovery of spontaneous beating and contractility during washout of calcium channel blocking drugs. For details of conditions see Table 2. Symbols for drugs are the same as in Figure 1.

Under these circumstances, concentration-effect curves for nifedipine remained reproducible for periods up to 3 h. By comparison, when nifedipine was superfused from unprotected syringes, approximately 50% of activity was lost in 1 h.

Reversibility of negative inotropic effects

In all cases, studies to determine the rate of recovery toward control of contractile states were performed after superfusion of the minimal drug concentration sufficient to abolish cell motion. Concentrations of drugs used in these experiments are summarized in Table 2. In all cases, washout of the calcium channel blocking drug was begun as soon as spontaneous contractions had ceased.

Rates of recovery of contractility varied widely among the six drugs examined. This is illustrated in Figures 2 and 3. For diltiazem, nifedipine and verapamil, recovery of contractility was essentially complete within 10 min, while after exposure to lidoflazine and perhexiline, recovery was far slower; D600 occupied an intermediate position. We noted that while there was no correlation between rates of recovery and the negative inotropic potency of the various agents examined, there was a relatively close correlation between the order of recovery rates and

Table 3 Effects of calcium channel blocking agents on the rapid phase of $^{45}Ca^{2+}$ uptake

Expt	n	*Control*	*Verapamil*	*D600*	*Diltiazem*	*Lidoflazine*	*Perhexiline*	*Nifedipine*
1	8	100 ± 3.4	84.3 ± 5.8		77.2 ± 5.2			96.9 ± 3.8
2	5	100 ± 6.9	75.7 ± 3.8				69.9 ± 9.8	
3	6	100 ± 5.7	83.7 ± 5.7	85.6 ± 3.7		83.7 ± 2.9		

Drug concentrations corresponded to EC_{90} values for negative inotropic effects. In all cases, mean effects ± s.e.mean are expressed in relation to the control ^{45}Ca uptake rate as 100%; rates of Ca uptake are from curves analyzed as described by Barry & Smith (1982). Mean control ^{45}Ca uptake rates expressed as absolute values ranged in individual cultures from 2.76 ± 0.19 to 5.41 ± 0.31 (s.e.mean) nmol mg^{-1} protein min^{-1}.

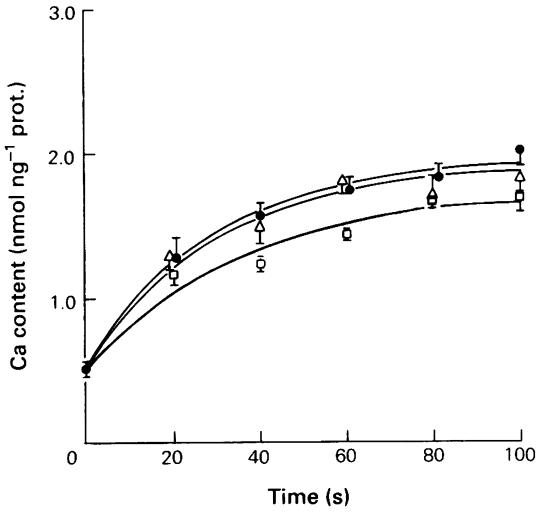

Figure 4 Effects of verapamil (□, 3.0×10^{-7}M) and nifedipine (△, 7.1×10^{-8}M) on the rapid phase of ^{45}Ca uptake, (●) control. Each point represents the mean of 20 determinations; vertical lines indicate s.e.mean.

that of the plasma half-lives of these agents, as estimated from available human studies in the literature (Table 2).

Effects on ^{45}Ca uptake

In order to examine the relation between negative inotropic effects and inhibition of ^{45}Ca uptake for representatives of several pharmacologically distinct classes of calcium channel blocking drugs, EC_{90} concentrations of drugs (Table 1) were used in all experiments and their effects on ^{45}Ca influx were compared with that of verapamil.

The result of an experiment comparing effects of nifedipine and verapamil is shown in Figure 4. In this experiment, verapamil significantly inhibited a component of the rapid uptake of Ca, whereas under these experimental conditions, we could not demonstrate inhibition of rapid Ca uptake by the dihydropyridine compound, nifedipine. Data from three experiments comparing effects of equipotent negative inotropic concentrations of calcium channel antagonists on calcium uptake are shown in Table 3. All significantly inhibited the uptake of ^{45}Ca relative to control by 15–30% ($P < 0.05$), with the exception of nifedipine. There was no significant difference in the extent of inhibition produced by verapamil, D600, lidoflazine, perhexiline, or diltiazem.

Discussion

The definition and characterization of calcium channel antagonists present a number of problems related to the heterogeneity of mechanisms of effects of calcium on target organs and to the possible actions of many of these drugs on calcium channel-independent mechanisms. All of the agents compared in this study have been characterized as calcium channel blocking drugs on the basis of their actions on myocardial electrophysiology. They can thus be distinguished from other drugs, such as sodium nitroprusside, nitroglycerine and diazoxide, which may inhibit calcium influx in vascular smooth muscle but not myocardium (Triggle & Swamy, 1980), or from cations such as Mn^{2+}, Co^{2+}, or La^{3+}, which compete directly with calcium at the cell surface (Hagiwara & Byerly, 1981). However, these drugs vary considerably in chemical structure (with the exception of verapamil and its methoxy-derivative, D600), electrophysiological effects, and accessory (that is, calcium channel-independent) actions. Representatives of each of the three classes of calcium channel blocking drugs designated Class I (1,4 dihydropyridines: nifedipine), Class II (phenylalkylamines: verapamil; D600), and Class III (diltiazem) by Glossman *et al.* (1982) were studied. The results of the present study indicate that there is considerable variability in potency, affinity of tissue binding, and effects on calcium influx among the six calcium channel blocking drugs examined in cultured myocardial cells.

The range of available methods for studying drug action on cardiac muscle for characterization of calcium channel blocking drugs has been reviewed by Nayler & Poole-Wilson (1981). Of previously utilized techniques, it ws concluded that selective inhibition of the calcium-dependent component of the action potential, using manoeuvres such as partial depolarization to inactivate the fast sodium current, represents the most specific means of identification of calcium channel blocking drugs. However, while qualitatively useful this technique is not well suited to quantitative studies. Alternative methods, such as those relying on reversibility of negative inotropic effects of drugs by added calcium lack specificity, while the theoretically desirable method of directly measuring drug effects on calcium influx has hitherto proved technically difficult.

Recent studies from this laboratory (Barry & Smith, 1982) have established the feasibility of both detecting and quantitating the effects of verapamil on the rapid phase of calcium influx into heart cells in tissue culture, with a threshold for detectable effects of verapamil of approximately 10^{-8}M. The present study uses this approach to compare the effects of six commonly used calcium channel antagonists on myocyte contractility and calcium influx, in order to

determine whether there is a constant relationship between the extent of inhibition of rapid calcium influx in this system and the negative inotropic effects of the agent concerned.

Comparison of the concentration-effect curves of these agents on myocyte contractility revealed substantial differences in potency. Nifedipine, lidoflazine and D600 exerted greater potency with respect to depressor effects than verapamil and diltiazem, while perhexiline was significantly less active than any of the other drugs examined (Table 1). There were no significant differences among the slopes of the concentration-effect curves for these agents after logit transformation, consistent with (but by no means proving) similarity of mechanism of action. In the cultured heart cell preparation used, there is little or no overt fast sodium channel activity (Shigenobu & Sperelakis, 1972), and agents such as quinidine exert minimal negative inotropic effects except at very high concentrations (Horowitz *et al.*, 1982). It is therefore unlikely that the inotropic effects of the six drugs examined here were influenced by actions of the fast sodium channel, especially at the drug concentrations used. While no comparison of the effects on myocardial preparations of the six drugs examined has previously been made, the relative negative inotropic effects observed are consistent with the results of more limited comparisons made by other investigators (Fleckenstein-Grün *et al.*, 1978; Triggle, 1981). However, the negative inotropic effects of any particular calcium channel antagonists in one myocardial preparation do not necessarily reflect its potency in all states or in all regions of the heart. For example, it is possible that verapamil is concentrated in the region of the atrioventricular node (Opie, 1980), potentially explaining its prominent negative dromotropic effects (Narimatsu & Taira, 1976).

A further possible complicating factor in comparisons of the negative inotropic potency of various calcium channel antagonists is the rate-dependency of such effects for verapamil and D600, but not for nifedipine (Bayer & Ehara, 1978). As none of the agents studied significantly affected beating rate in this preparation (Barry & Smith, 1982), rate-dependent negative inotropic effects would not have affected the concentration-effect curves for the agents examined here. However, this might limit the predictive value of our results for relative potency of these drugs in other myocardial preparations.

In some other tissues, the time of onset of maximal effects of calcium channel antagonists has been observed to be far slower (Bleeker & van Zwieten, 1978; Vanhoutte, 1982) than indicated by our observations. While it has been suggested (Bleeker & van Zwieten, 1978) that this may reflect intracellular actions of some of the drugs, it is also possible that the shorter equilibration time for peak negative inotropic effects observed in the current study reflects the minimal diffusion barriers in the preparation used. The minor differences in equilibration time among the various agents examined may be due at least in part to variations in lipid solubility rather than different sites of action.

The effects of inotropically equipotent concentrations of drugs on the rapid phase of ^{45}Ca influx into heart cells were examined in order to determine whether all of these agents, like verapamil (Barry & Smith, 1982), inhibit a component of this flux. This fundamental effect of calcium channel antagonists could be demonstrated with each of the agents examined except nifedipine. Concentrations of verapamil, D600, lidoflazine, diltiazem and perhexiline associated with a 90% reduction in amplitude of myocyte contraction produced similar degrees of inhibition of ^{45}Ca influx (Table 3). This is consistent with the results of Morgan *et al.* (1983), who noted similar effects on the Ca^{2+} transient in aequorin-injected cat papillary muscles at negative inotropic concentrations of calcium channel antagonists. On the other hand, nifedipine exerted a smaller and statistically insignificant effect on ^{45}Ca influx in our experiments. Because this finding was initially surprising to us, we repeated the experiment three more times and in each instance found no significant effect of nifedipine on ^{45}Ca influx at the concentration (7.1×10^{-8}M) producing 90% inhibition of contractile amplitude, while on each occasion observing between 21 and 25% inhibition of ^{45}Ca influx at 60 s by 3×10^{-7}M verapamil. These results are consistent with the recently reported findings of Pang & Sperelakis (1983) and suggest possible differences in the mechanism of negative inotropic action of this calcium channel antagonist compared to verapamil. Verapamil and D600 also alter Na influx, presumably via a 'slow' Na channel (Galper & Catterall, 1980); this could decrease Na loading and thus decrease Ca influx via Na-Ca exchange (Barry & Smith, 1982) in addition to decreasing slow calcium channel Ca influx. It is likely that the dihydropyridine subgroup of calcium channel antagonists interacts with sarcolemmal calcium channels at a different site from that affected by verapamil (Antman *et al.*, 1980; Kass, 1983; Marsh *et al.*, 1983). Nifedipine may also inhibit calcium sequestration by sarcoplasmic reticulum (Colvin *et al.*, 1981). The recent report of Williams & Jones (1983) indicating high-affinity binding sites for a dihydropyridine calcium channel antagonist in myocardial subcellular fractions enriched in sarcoplasmic reticulum rather than sarcolemma is of interest in this regard. It is unlikely that loss of nifedipine potency occurred due to exposure to light during ^{45}Ca flux studies since we took special precautions to minimize such exposure and the negative inotropic effect of the drug was preserved under these experimental conditions.

The six drugs examined differed markedly regarding the speed of recovery of spontaneous beating and contractility after drug superfusion ceased (Table 2). Since the washout studies were initiated at steady state conditions of drug-calcium channel interaction, the rate of reversibility of inotropic effects is likely to reflect both the affinity of a given drug for the calcium channels and the nature and extent of cellular distribution of each agent. There was a reasonably close correlation between rate of loss of pharmacological effect after removal of these drugs from cultured heart cells and the plasma half-lives of the drugs, in so far as such information is available (Table 2). This finding raises the possibility that the extent of drug binding to myocardium may be representative of general tissue drug distribution, which is a direct determinant of half-life.

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